ENGLISH TRANSLATION OF THE ANNEX TO THE IPER

Success has also been achieved in extracting a corresponding enzyme from defatted cottonseed meal.

In addition to plant sources, microorganisms also serve as a source, whereas, in particular, corynebacteria (Corynebacterium ovis), Escherichia coli, baker's yeast cells and streptomycetes (Streptomyces hachijoensis) are to be mentioned.

However, it has also been possible to isolate phospholipase from mammalian cells, for example from human eosinophils and rat brain microsomes.

The known phospholipase D enzymes present a heterogeneous picture in regard to their molecular weights:

Thus, the soluble enzyme isolated from cotton seed has a molecular weight of 71 000 ± 3000 Da; phospholipase D from peanut seeds has a molecular weight of 200 000 ± 10 000 Da and PLD from human eosinophils has a molecular weight of approx. 60 000 Da.

Corresponding bacterial enzymes, as can be isolated, for example, from Corynebacterium ovis, possess a molecular weight of approximately 90 000 Da.

With regard to the isoelectric point, phospholipase D from peanut seeds is known to have pI values of 4.65 while, on the other hand, the pI of a crude extract from human eosinophils is between 4.8 and 5.0 and, as a result of additional purification, can reach a value of between 5.8 and 6.2.

R. Lambrecht et al. ("A facile purification procedure of phospholipase D from cabbage and its

characterization"; (1992) Biol. Chem. Hoppe Seyler Vol. 373 (2) 81-88) describe purification of PLD from white cabbage in two steps. This method comprises an ammonium sulfate precipitation and a subsequent Ca^{+}-mediated affinity chromatography.

The publication by I. Schäffner et al. ("Genomic structure, cloning and expression of two phospholipase D isoenzymes from white cabbage", Eur. J. Lipid Sci. Technol. 104, 79-87 (2002); corresponding to dissertation (2001)) describes how recombinant phospholipase D-active isoenzymes can be obtained from white cabbage using cloning methods and how these isoenzymes can be characterized in regard to their specific hydrolysis activity in dependence on the pH and on the Ca^{2+} concentration as well as in regard to their transphosphatidylating properties.

The review article by Michael Heller in Advanced Lipid Research, 1978, Volume 16, pages 267 to 326, provides a general overview of the state of knowledge with regard to phospholipase D.

In "Identification of two isoenzymes of phospholipase D from opium poppy" (Direct submission (2001) NCBI GenBank, accession Nos. AAL48261-AAL48264 and multiple sequence comparison), A. Lerchner et al. describe two truncated phospholipase D1 polypeptides, as well as two other truncated phospholipase D2 polypeptides, from Papaver somniferum. As can be seen from the multiple sequence comparison, the part amino acid sequences of proteins D1 and D2 exhibit a sequence identity of 98% to each other. In addition, the part sequences which are described possess a high degree of homology (70-84%) with the well-characterized phospholipase D varieties of the α type. However, since the sequence determination is not complete at the 5' end, it is not possible to assign the phospholipase D1 and D2 polypeptides to a defined enzyme.

activity optimum at pH values between 5.0 and 6.0.

A defined value for the isoelectric point can be obtained by subjecting isolated fractions to further purification.

For this reason, the protein fraction of the present invention is also, in particular, characterized by the fact that the subfraction A has an isoelectric point pI of 8.7 and a molecular mass of 116.4 kDa as well as a hydrolytic activity optimum at pH 8.0. The corresponding preferred values for subfraction B are 114.1 kDa with regard to the molecular mass and 6.7 with regard to the isoelectric point pI, with the hydrolytic activity optimum being at pH 5.5. These features are also encompassed by the present invention.

As already mentioned, protein fractions possessing phospholipase D activity are usually calcium ion-dependent. However, this pronounced dependence has not proved to be true in the case of the claimed plant protein fraction from Papaveraceae, which is imperatively Zn^{2+} ion-activatable. However, the activity optimum of this protein fraction can also be reached in the presence of calcium ion concentrations, which are then usually between 40 µM and 100 mM, with corresponding enzyme activities appearing at concentrations of between 2 and 20 mM and between 5 and 15 mM.

With regard to subfraction B, the present invention claims a protein variant whose activatability optimum occurs in the presence of Zn^{2+} ion concentrations which are between 1.0 and 10 mM and, particularly preferably, at 5 mM.

As what is important for the invention, inter alia, the present invention provides for subfractions A and/or B to possess carbohydrate moieties such that they are consequently present in glycosylated form as N-linked

glycoproteins, and

AMENDED SHEET